Conifer Associates and Mycorrhizal Syntheses of Some Pacific Northwest Suillus Species

BY
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Abstract. The relative frequency of occurrence of sporophores of various *Suillus* species, as determined in natural stands of certain conifers in northern Idaho, indicated possible mycorrhizal relationships among: *S. cavipes* and *S. grevillei* with *Larix occidentalis;* and *S. granulatus*, *S. sibiricus*, *S. tomentosus* vars. *discolor* and *tomentosus* with *Pinus monticola*. Seedlings of *L. occidentalis*, *P. contorta*, *P. ponderosa*, and *Pseudotsuga menziesii* were grown aseptically and inoculated with pure cultures of seven species and varieties of *Suillus*. Mycorrhizae were formed by *S. brevipes*, *S. pallidiceps*, and *S. tomentosus* var. *tomentosus* with *P. contorta* and *P. ponderosa; S. tomentosus* var. *discolor* with *P. ponderosa;* and *S. grevillei* with *P. menziesii. Suillus grevillei* stimulated dichotomous branching of short roots of *P. ponderosa*, but no mycorrhizal relationship was formed.

COMPARATIVELY FEW of the fungi that form ectotrophic mycorrhizae with various species of trees in nature have been positively identified. Direct isolations from mycorrhizae often have failed because of difficulties in obtaining pure cultures of the fungi and in identifying them since they rarely fruit in culture. Two indirect methods have been employed but each, used separately, has one or more critical limitations. Analysis of the frequency of sporophores of fungi in constant proximity to certain species of trees involves the assumption that occurrence of sporulation is related to the presence of mycorrhizae. The other method, synthesis of mycorrhizae in pure culture, seldom simulates natural conditions. The failure of a fungus to form mycorrhizae under such conditions cannot necessarily eliminate the possibility that formation of mycorrhizae does occur in nature. Both methods, used together, complement each other and provide stronger evidence on specific tree-fungus associations.

Fungi that have been associated with ectotrophic mycorrhizae of conifers were summarized by Trappe (1962). Most of these associations have been based on the occurrence of sporophores near certain species of trees. Various species of Boletaceae form mycorrhizae with many conifers (Singer 1962). Within this family, the genus *Suillus* (Smith and Thiers 1964) has received the most attention in recent years. Several species of *Suillus* have been demonstrated to form mycorrhizae with

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certain conifers in pure culture (Doak 1934; Vozzo and Hacskaylo 1961; Bryan and Zak 1961, 1962; Volkart 1964; Marx and Zak 1965; Trappe 1967).

Species of *Suillus* associated with conifers have been reported by Snell (1936, 1945), Snell and Dick (1941, 1958, 1961), Singer (1945), Snell *et al.* (1959), Dick (1960), Dick and Snell (1960, 1965), Smith and Thiers (1964), and Smith *et al.* (1965). These studies emphasized taxonomy of the fungi rather than the capacity to form mycorrhizae. Slipp and Snell (1944) reported on studies oriented directly toward observations of bolete sporophores associated with specific conifers, and Cooke (1955) reported several bolete species consistently found with specific conifer associations. Both studies were conducted in eastern Washington and adjacent Idaho.

Synthesis of mycorrhizae in pure culture was first used by Melin in 1921 to confirm the capacity of suspected fungi to form mycorrhizae with selected species of trees. Since then, Melin's technique has been modified by various investigators (Norkrans 1949, Hacskaylo 1953, Marx and Zak 1965) and used extensively to confirm suspected mycorrhizal relations.

This study was made to determine: (1) the frequency of occurrence of sporophores of certain species of *Suillus* with specific conifers in natural stands of mixed conifers, and (2) which among the species of *Suillus* observed formed mycorrhizae when grown together with certain conifers in pure culture.

Methods and Procedures

Habitat studies. Habitat studies were conducted in the fall of 1966 in the Kaniksu National Forest, Bonner Co., Idaho, in the vicinity of Priest Lake, Upper Priest Lake, and the Priest River Experimental Forest. Because of the very dry season, sporophores were found almost exclusively on moist sites.

All observational data were obtained in the *Thuja-Tsuga/Pachistima* Habitat Type (Daubemire 1953). Judging from the seral trees present, none of the stands visited were climax. Seral conifers encountered were: *Pinus monticola* Dougl. and *Larix occidentalis* Nutt., which were consistent components of the habitat type; *Abies grandis* (Dougl.) Lindl. and *Pseudotsuga menziesii* (Mirb.) Franco, which were often found; and *Pinus contorta* Dougl. and *P. ponderosa* Dougl. which occurred occasionally. *Thuja plicata* Donn. and *Tsuga heterophylla* (Raf.) Sargo, the climax conifers, were also present in all stands.

Conifer associates within a radius of 100 feet of *Suillus* sporophores were tallied in the field and their distances recorded in feet. The 100-foot radius was selected arbitrarily to include the bulk of root systems of trees that might be involved in a mycorrhizal relationship. Identification of most *Suillus* species was verified by A. H. Smith or H. D. Thiers.

Formation of mycorrhizae in pure culture. The procedure recommended by Hacskaylo (1953) and modified by Marx and Zak (1965) was used in the attempts to synthesize mycorrhizae in the laboratory. Sixty ml of peat moss ground in a Wiley Mill to pass through a No. 30 mesh screen were added to 840 ml of vermiculite and mixed thoroughly in a 2-liter Erlemyer flask; 1-liter flasks received half this amount. Prior to inoculation, 600 ml of Melin's nutrient medium (1921) as modified by Norkrans (1949) were added to each 2-liter flask and 300 ml to each 1-liter flask. Flasks were autoclaved for 30 minutes at 121°C and 18 psi, cooled for 24 hours, and reautoclaved for 30 minutes. Final pH varied between 5.0 and 5.4.

Cultures were obtained by direct isolation from sporophores and plated on modified Hagem nutrient agar medium (Modess 1941); then small blocks of mycelium were transferred and grown

in 250-ml flasks containing 75 ml of modified Hagem broth. Cultures were agitated on a Gyrotory shaker at 140 rpm, and then homogenized in a Waring Blendor for 1 minute at low speed. Ten ml of the homogenized liquid culture were used to inoculate 1- and 2-liter flasks 1 week prior to introduction of germinating conifer seed. Uninoculate flasks served as controls.

Fungi used were: *Suillus brevipes* (Pk.) Kuntze (WSP 55324,[1] Washington); *S. grevillei* (Klotzsch) Sing.; *S. pallidiceps* A. H. Sm. and Thiers (WSP 55320, Washington); *S. tomentosus* (Kauff.) Sing. var. *tomentosus* (WSP 55322, Idaho; and Trappe 399, Oregon); *S. tomentosus* var. *discolor* A. H. Sm., Thiers, and Miller (Miller 2714, Idaho); *S. lakei* (Murr.) A. H. Sm. and Thiers var. *lakei* (WSP 55319, Idaho); *S. lakei* var. *pseudopictus* A. H. Sm. and Thiers (Trappe 368, Oregon). Two isolates of *S. tomentosus* var. *tomentosus* from different locations and two varieties of *S. tomentosus* and *S. lakei* were used to determine possible differences in capacity to form mycorrhizae. Two series of inoculations were made. Series 1 paired *Suillus* species and conifers reported to form mycorrhizae in nature. Series 2 paired *Suillus* species with conifers not previously reported to be natural mycorrhizal partners.

Seeds of *Larix occidentalis*, *Pinus contorta*, *P. ponderosa*, and *Pseudotsuga menziesii* were stratified in wet sand at 5°C for 2 weeks, washed in running tap water for 30 minutes, then placed in 100 ml of distilled water with several drops of Tween 20 (polyoxyethylene sorbitan monolaurate) and agitated for 15 minutes. Seeds were surface-sterilized with 30.2-percent hydrogen peroxide according to the technique of Trappe (1961); *P. ponderosa* seeds were treated for 1 hour and seeds of the other 3 species for 30 minutes.

Surface-sterilized seeds were placed directly on modified Hagem medium in petri plates (10 to 20 seeds per plate). Germinating seeds with radicles approximately 1 cm long were removed from the petri plate and implanted in the prepared flasks, 3 seeds per flask. Pieces of vermiculite were removed from inoculated flasks 2 weeks after planting and placed on modified Hagem medium to assess cultural purity.

Three to 6 months were provided for mycorrhizal development. Two growth chambers were employed: a built-in chamber with a light intensity of 1,200 foot-candles and a Sherer Model Cel 512-37 plant growth chamber with 2,560 foot-candle intensity. The former was programmed to run on a 12-hour light cycle at a constant temperature of 23°C. The latter chamber was programmed for a 12-hour light cycle at a temperature of 23°C during the light period and 12°C during the dark period.

After 3 to 6 months roots were harvested, fixed in FAA (Sass 1951), and stored for sectioning. Apparent mycorrhizae were removed from FAA, washed in tap water for 5 minutes, embedded in 5-percent water agar blocks, and sectioned on an International Cryostat microtome at 16 microns. Sections were stained with dilute safranin (7 ml of 1-percent solution of safranin in 95-percent ethanol diluted with 100 ml of distilled water) and examined for presence and development of the fungal mantle and Hartig net.

Results

Habitat studies. Sporophores of 8 species and 2 varieties of *Suillus* were observed within 100 feet of specific conifers in the *Thuja-Tsuga/Pachistima* Habitat Type. As stated previously, *Tsuga heterophylla* and *Thuja plicata* were consistently present in the stands; and sporophores

[1] WSP numbers are accession numbers of the Mycological Herbarium, Dept. of Plant Pathology, Wash. State Univ., Pullman. Other numbers are the personal collection numbers of the collectors involved.

were often found within 100 feet of them. *Suillus cavipes* (Opat.) A. H. Sm. and Thiers and *S. grevillei* were consistently associated with *Larix occidentalis* whereas *S. granulatus* (Fr. Kuntze), *S. sibiricus* (Sing.) Sing., and *S. tomentosus* vars. *discolor* and *tomentosus* were consistently associated with *Pinus monticola* (Table 1). *S. albidipes* (Pk.) Sing., *S. lakei* vars. *pseudopictus* and *lakei* and *S. subolivaceus* A. H. Sm. and Thiers were not observed frequently enough to establish association with specific conifers. These observations confirm the observations of Smith and Thiers (1964) and Smith *et al.* (1965) of sporophores of *S. cavipes* and *S. grevillei* associated with *L. occidentalis*, and *S. sibiricus* and *S. granulatus* with *P. monticola*. The occurrence of *S. tomentosus* var. *discolor* with *P. monticola* likewise was confirmed in this study. Smith *et al.* (1965) suggest *P. monticola* might be a conifer associate. Interestingly in 17 of 18 observations *S. tomentosus* var. *tomentosus* occurred within 50 feet of *P. monticola*. This was the only conifer, except *Tsuga heterophylla* and *Thuja plicata*, that sporophores of *S. tomentosus* var. *tomentosus* occurred with consistently. *Pinus contorta*, the conifer associate listed by Smith and Thiers (1964) and by Slipp and Snell (1944), was recorded only once.

Pure culture syntheses of mycorrhizae. Table 2 summarizes the results of inoculating 4 conifer species with selected *Suillus* species. *Suillus tomentosus* var. *tomentosus* formed mycorrhizae with *Pinus ponderosa* and *P. contorta* but not *Larix occidentalis; Pseudotsuga menziesii* was not inoculated. *Suillus tomentosus* var. *discolor* formed mycorrhizae with *P. ponderosa* only. *Suillus brevipes* and *S. pallidiceps* formed mycorrhizae with *P. ponderosa* and *P. contorta* but not with *P. menziesii* or *L. occidentalis*. *Suillus grevillei* formed mycorrhizae with *P. menziesii* only. Both varieties of *S. lakei* failed to form mycorrhizae with *P. menziesii*, the only conifer inoculated with these isolates.

There appeared to be no difference in mycorrhiza-forming ability between isolates of *Suillus tomentosus* var. *tomentosus* from Oregon and Idaho. Both isolates formed morphologically similar mycorrhizae with *Pinus contorta* and *P. ponderosa*.

TABLE 1. Suillus-conifer associations observed in the Thuja-Tsuga/Pachistima Habitat Type in the Kaniksu National Forest, vicinity of Priest Lake, Bonner County, Idaho.

		No. of observations of *Suillus* when the given tree species was found within 50 and 100 feet[1]											
	Total no. of observ. of	*Pinus monticola*		*Larix occidentalis*		*Pseudotsuga menziesii*		*Abies grandis*		*Pinus contorta*		*Pinus ponderosa*	
Suillus spp.	*Suillus*	50	100	50	100	50	100	50	100	50	100	50	100
S. cavipes	60	41	45	52	58	27	30	25	25	5	5	2	2
S. grevillei	43	28	30	40	42	18	18	14	15	1	1	0	0
S. sibiricus	33	29	30	6	12	4	4	7	10	3	4	0	0
S. granulatus	24	23	23	8	10	0	4	5	6	0	0	0	0
S. tomentosus var. *discolor*	20	19	19	5	8	8	8	5	5	1	1	1	1
S. tomentosus var. *tomentosus*	18	17	17	12	12	2	2	4	5	1	1	1	1

[1] Example of how to interpret this table: *S. cavipes* was observed at 60 locations (first data column). At 41 of those locations (second data column) one or more *Pinus monticola* trees were found within 50 feet.

TABLE 2. Results of attempts to synthesize mycorrhizae in pure culture.

Suillus species	*Pinus ponderosa*		*Pinus contorta*		*Pseudotsuga menziesii*		*Larix occidentalis*	
	No. trees	No. with mycorrhizae	No. trees	No. with mycorrhizae	No. trees	No. with mycorrhizae	No. tress	No. with mycorrhizae
S. brevipes	33	27	19	17	7	0	7	0
S. pallidiceps	13	10	8	7	7	0	6	0
S. grevillei	7	0	5	0	6	5	18	0
S. tomentosus var. *discolor*	6	6	8	0	6	0	7	0
S. tomentosus var. *tomentosus* Idaho[1]	23	21	12	12	—	—	7	0
S. tomentosus var. *tomentosus* Oregon[1]	14	9	11	9	—	—	—	—
S. lakei var. *lakei* Idaho[1]	—	—	—	—	22	0	—	—
S. lakei var. *pseudopictus* Oregon[1]	—	—	—	—	12	0	—	—

[1] A dash in the data columns means that no inoculations were made.

Mycorrhizae formed in pure culture are characterized in Table 3. Four types of mycorrhizae, all ectotrophic, were recorded. The first or simple type was club-shaped, noticeably thicker in diameter and shorter than uninfected roots, and without root hairs. Except for those formed by *Suillus pallidiceps* with *Pinus contorta* and *P. ponderosa*, some simple mycorrhizae were formed in all cases; *S. grevillei* with *Pseudotsuga menziesii* formed only simple mycorrhizae.

The second or bifurcate type, resulting from the dichotomous branching of the infected root into equal branches, was the most common type encountered. It was present in various numbers in all cases except the mycorrhizae formed by *Suillus grevillei* with *Pseudotsuga menziesii*. Root hairs were usually absent but occasionally were present at the base of the short root at its point of attachment to the lateral root. The bifurcate type was thicker and shorter than an uninfected root.

The third or double-bifurcate type resulted from the dichotomous branching of the infected root into two equal branches with the two branches then dichotomously branching into two more equal branches. No root hairs were observed. The double-bifurcate type was noticeably thicker than, but often attained the length of, uninfected roots. This type was commonly encountered in all mycorrhizae except *Suillus brevipes* with *Pinus ponderosa* and *S. grevillei* with *Pseudotsuga menziesii*. This type appeared to be intermediate between the bifurcate and coralloid types. However, developmental sequences of mycorrhizae were not investigated and the double-bifurcate type may represent an intermediate stage rather than a final stage of development. Longer developmental time may have resulted in the double-bifurcate type developing into a coralloid type of mycorrhiza.

The fourth or coralloid form, resulting from repeated dichotomous branching of the infected root, was the least frequent. This type was observed in mycorrhizae formed by *Suillus brevipes*, *S. tomentosus* var. *tomentosus*, and *S. pallidiceps* with *P. contorta* and *P. ponderosa*.

Three fungal mantles were observed. The first exhibited a rather thick, loosely arranged layer of hyphae in which individual hyphae retained their identity. This type was formed by *Suillus tomentosus* vars. *tomentosus* and *discolor* with *Pinus ponderosa* (Table 3). The second

TABLE 3. Characteristics of mycorrhizae synthesized in pure culture.

Suillus species	Conifer species	Type	Mantle thickness (μ)	Hartig net development[1]
S. brevipes	*Pinus ponderosa*	Simple Bifurcate Coralloid	22–28	Good
	P. contorta	Simple Bifurcate Quadrifurcate Coralloid	30–60	Fair-good
S. grevillei	*Pseudotsuga menziesii*	Simple	42–65	Good
S. pallidiceps	*P. ponderosa*	Bifurcate Quadrifurcate Coralloid	18–28	Fair
	P. contorta	Bifurcate Quadrifurcate Coralloid	10–18	Poor
S. tomentosus var. *discolor*	*P. ponderosa*	Simple Bifurcate Quadrifurcate	33–77	Good
S. tomentosus var. *tomentosus*, Idaho	*P. ponderosa*	Simple Bifurcate Quadrifurcate Coralloid	35–60	Good
	P. contorta	Simple Bifurcate Quadrifurcate	14–22	Fair
S. tomentosus var. *tomentosus*, Oregon	*P. ponderosa*	Simple Bifurcate Quadrifurcate	55–66	Fair-good
	P. contorta	Simple Bifurcate Quadrifurcate Coralloid	14–22	Fair-good

[1] Good = Hartig net developed to a depth of 3 or more cortical cells.
Fair = Hartig net developed to a depth of 2 cortical cells.
Poor = Hartig net developed to a depth of 1 cortical cell or less.

type was similar to the first but was thinner and more compact; individual hyphae could be distinguished. This second type was formed by *S. tomentosus* var. *tomentosus* and *S. pallidiceps* with *P. contorta* and *P. ponderosa*, and by *S. brevipes* with *P. contorta*. The third type possessed a thick layer of hyphae in which individual hyphae could not be distinguished. A cross section through the mycorrhiza showed a pseudoparenchymatous structure. This type was found in mycorrhizae formed by *S. grevillei* with *P. menziesii* and *S. brevipes* with *P. ponderosa*.

The Hartig net was usually well developed, but mycorrhizae formed by *Suillus pallidiceps* with *Pinus contorta* consistently exhibited poor development of the Hartig net (Table 3). The Hartig net was developed to a depth of at least 2 cortical cells for all other mycorrhizae; and in *S. grevillei* with *Pseudotsuga menziesii* and *S. brevipes* and *S. tomentosus* vars. *tomentosus* and *discolor* with

P. ponderosa the entire cortical system was involved.

Suillus grevillei stimulated dichotomous branching of short roots of *Pinus ponderosa*. Bifurcate and quadrifurcate types of branching were present. No fungal mantle or Hartig net was observed, indicating lack of formation of mycorrhizae.

Discussion

Judging by constant associations in nature, Trappe (1963), Smith and Thiers (1964), and Smith *et al.* (1965) regard *Suillus brevipes*, *S. pallidiceps* and *S. tomentosus* var. *tomentosus* as possibly forming mycorrhizae with 2- and 3-needle pines. The ability of *S. brevipes*, *S. pallidiceps*, and *S. tomentosus* var. *tomentosus* to form mycorrhizae with *Pinus ponderosa* and *P. contorta* in pure culture was demonstrated in the present study for the first time.

Suillus tomentosus var. *discolor* produced mycorrhizae with *Pinus ponderosa*, a conifer not indicated as an associate by observational data (Table 1). However, sites supporting *P. ponderosa* were too dry in the fall of 1966 to provide additional data on the fruiting of this fungus in relation to the conifer.

The production of mycorrhizae by *Suillus grevillei* with *Pseudotsuga menziesii* was unexpected. *Suillus grevillei* has been considered mycorrhizal only with *Larix* species (Trappe 1962, Smith and Thiers 1964, Smith *et al.* 1965); although two reports (Schwitzer 1930, Rayner 1938) indicate possible mycorrhizal relationships with *Pinus elliottii* Engelm., *P. sylvestris* L., and P. *taeda* L. *Suillus grevillei* failed to form mycorrhizae with *L. occidentalis* in this study, but mycorrhizae have been produced in pure culture by *S. grevillei* with *Larix* (Melin 1925). The production of mycorrhizae by *S. grevillei* with *P. menziesii* suggests that the fungus has the capacity to form mycorrhizae with several hosts. Perhaps fruiting of the fungus results only from formation of mycorrhizae with specific hosts. However, this possibility was not indicated by other inoculations of conifers of *Suillus* species (Table 3). *Suillus brevipes*, *S. pallidiceps*, *S. tomentosus* vars. *discolor* and *tomentosus*, and *S. grevillei* (except as previously noted) failed to synthesize mycorrhizae with conifers not reported to form mycorrhizae with them in nature.

The reason that *Suillus lakei* vars. *lakei* and *pseudopictus* failed to form mycorrhizae with *Pseudotsuga menziesii*, a constant associate in nature, is unknown. It was noted that *P. menziesii* failed to form abundant short roots in pure culture. In some instances short roots were entirely absent, but the reason for this absence is likewise unknown. When *P. menziesii* was inoculated with *S. grevillei*, short roots as well as mycorrhizae were produced; however, the number of short roots produced was few. Uninoculated controls also failed to form abundant short roots, indicating that the presence of the fungus was apparently not the cause. Temperatures and daylengths as well as the other artificial conditions under which the seedlings were grown—i.e. rooting medium, nutrient solution, and enclosure in glass flasks—may have affected production of short roots.

The dichotomous branching of short roots of *Pinus ponderosa* when inoculated with *Suillus grevillei* illustrates the importance of microscopic examination to determine if a true mycorrhizal relationship has been established. Forking has been observed in pine roots by Levisohn (1952) and Slankis (1948, 1949, 1950, 1961). Slankis thinks forking was stimulated by the production of growth substances such as α-indoleacetic acid, β-naphthaleneacetic acid, or other byproducts of metabolism by mycorrhizal fungi. Microscopic examination of the forked roots showed no fungal mantle or Hartig net. Possibly exudates or byproducts of metabolism from *S. grevillei* stimulated the dichotomous branching of

P. ponderosa short roots in this study. Branching was not stimulated in other inoculations made to nonassociated coniferous hosts.

Results of this study demonstrate that *Suillus tomentosus* vars. *discolor and tomentosus*, *S. brevipes*, *S. pallidiceps*, and *S. grevillei* can form mycorrhizae in pure culture with one or several species of conifers. Production of mycorrhizae by *S. grevillei* with *Pseudotsuga menziesii* indicates that the absence of sporophores in the vicinity of a tree does not preclude possible formation of mycorrhizae by the fungus with that species. The stimulation of dichotomous branching of short roots by *Pinus ponderosa* grown in the presence of *S. grevillei* emphasizes the importance of microscopic examination of short roots to determine presence of a fungal mantle and Hartig net.

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